EPIDEMIOLOGY, STATISTICS and PSYCHOSOMATIC NO. 623

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE
Dr. Little, Chm.
Dr. Cattell
Dr. Jacobson

Application For Research Grant

Date: June 30, 1967

1. Name of Investigator(s): (include Title and Degrees)

Walter B. Essman, Ph.D. - Professor

2. Institution & Address:

Queens College of the City University of New York
Kissena Blvd.
Flushing, N. Y. 11367

3. Short Title of Project:

Studies of Nicotine Action upon Memory Consolidation

4. Proposed Starting Date: October 1, 1967

5. Anticipated Duration of this Specific Study: Two years.

6. Brief Descripton of Objectives or Specific Aims:

The major objective of this proposed research is to assess the possible role of nicotine in determining the temporal character of memory consolidation in experimental animals. The proposed experiments represent an attempt to relate the effects of nicotine in the central nervous system to the behavioral processes which are dependent upon such central events. Specifically, the effect of nicotine on brain amine concentration and turnover will be related to the process of memory consolidation (the process by which the memory trace becomes fixated in the central nervous system; this may be empirically defined in terms of the time interval following learning within which memory for an acquired event may be disrupted by the introduction of external agents and/or events). Since the previous work in this laboratory has demonstrated that the temporal course for memory consolidation may be shortened or lengthened, depending upon changes brought about in brain amine levels, specifically serotonin, the studies proposed herein are based upon the assumption that nicotine-induced changes in brain amine levels will constitute the basis upon which memory consolidation may be facilitated. The parameters to be considered in the proposed studies are nicotine dosage and the intensity of the amnesic event which, for the present experiments, will be electroconvulsive shock. A one-trial conditioning technique will be utilized to establish a passive avoidance response, the retention of which will be tested, based upon the interaction of the effects of nicotine on the central nervous system and the effects of electroconvulsive shock both on the central nervous system as well as an amnesic event specific to the behavior concerned in the proposed studies.

7. Give a Brief Statement of your Working Hypothesis: Memory consolidation, as defined by the post-conditioning time interval, within which retention of a conditioned response may be disrupted by electroconvulsive shock, will be facilitated through the prior administration of nicotine. Such facilitation will be defined by a reduction in the time interval following learning, wherein electroconvulsive shock results in a subsequent retrograde amnesia for the conditioned behavior.

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8. Details of Experimental Design and Procedures: (Attach Separate Pages)

See attached pages 5, 6, 7.

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

A large air-conditioned laboratory with adjoining animal housing facilities is available and completely equipped with both behavioral testing apparatus and necessary equipment for biochemical procedures.

10. Additional Requirements:

None

11. Biographical sketches of all principal and professional personnel (append)

See attached page 10.

12. List of publications: (Five most recent as pertinent) (append)

See attached page 11.

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13. Budget: (1st year)

	% time	Amount
A. Salaries (Personnel by names)		
Professional		
Technical		
Full-Time Research Assistant (To be selected)	100	R
Part-Time Research Assistant (To be selected)	50	R
Sub-Total		R
B. Consumable Supplies (list by categories)		
Animal purchases		1,200.
Animal food, bedding and cleaning supplies		600.
Chemicals and glassware		600.
Disposable cages		300.
Sub-Total		2,700.
C. Other Expenses (itemize)		
Photographic reproduction and duplication costs		200.
Reprint costs		300.
Sub-Total		500.
D. Permanent Equipment (itemize)		
E. Overhead (15% of A+B+C)		1,770
Total		$13,570

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	$2,500	$500		$1,800	$13,800
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Walter B. Casman
Director of Project

Signature [illegible]
Business Officer of the Institution

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Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

	Title of Project	Source	Amount	Duration
Current	Psychophysiological Properties of Thermal Change	National Institute of Mental Health	$20,050	6/1/66-12/31/67
	Role of Serotonin in Memory Consolidation	National Institute of Mental Health	4,200	9/1/66-8/31/67
Pending	Purine Metabolites in Memory Formation	National Institute of Mental Health	4,200	8/1/67-7/31/68

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8. Details of Experimental Design and Procedures:

All experiments will be based upon the use of a reliable technique which provides for the establishment of a conditioned passive avoidance response in mice, utilizing a single training trial (Essman & Alpern, 1965). The apparatus basically consisted of a small transparent lucite chamber fixed to a hole opening into a larger opaque chamber. The floor of the opaque chamber consisted of stainless steel grids which were wired in series through a cam-operated grid scrambler to a 95 volt power supply. Completion of the circuit by an animal stepping from the outer chamber into the inner one, through any 2 adjacent grids, provided for a 3.2 ma. shock delivered through the paws of the animal for 1 sec. The interval between placement of the animal in the outer chamber and its entry into the larger adjacent chamber was measured (response latency).

All animals utilized in the proposed experiments will be male, CF-1 strain mice (Mus Musculus), to be obtained from a commercial vendor (Carworth Farms, Inc.) at weaning (21 days), and following adaptation to conditions of laboratory housing (10 animals per cage), will be utilized as experimental subjects at approximately 30 days of age. They will eat each other up

The specific experiments will be concerned with:

1. Dose-response relationships for nicotine sulfate and rate of memory consolidation; the latter will be determined from the incidence of reduced retrograde amnesia induced by electroconvulsive shock (ECS).
2. The temporal gradient for ECS-induced retrograde amnesia in animals pretreated with nicotine sulfate.
3. The effect of nicotine sulfate on the incidence of conditioned response acquisition as a function of conditioning stimulus intensity.
4. The effect of nicotine sulfate on the incidence of conditioned response retention as a function of the intensity of the amnesic stimulus (ECS).

In the first series of experiments nicotine sulfate will be administered i.p. to mice in dose ranges of 0.25 mg/kg to 2.00 mg/kg (larger doses would not be indicated in view of neurotoxic effects). Control groups will be given an equivalent i.p. volume of 0.9% saline. Each group will be given a single training trial one hr. post-injection (stable CNS stimulant effect reached) in the apparatus described above, utilizing a 3.0 mA, 3 sec. training shock (yielding approximately 100% conditioned response acquisition), followed either immediately, 5, 10, 15, 20, 30, 45, or 60 min. by a 10 mA electroconvulsive shock (ECS), applied transcorneally for 200 msc. (sufficient to produce approximately 100% retrograde amnesia in control mice, when applied immediately post-training). A testing trial will be given to all groups of mice 24 hrs. following the training trial. Conditioned response retention will be evaluated on the basis of response latencies in the testing trial ($L \geq 30$ sec.) and retrograde amnesia will also be defined in terms of the absence of conditioned response as determined from latency measures ($L \leq 5$ sec.). Control groups, both drug and saline-treated, as described above, will be given no conditioning shock followed at the appropriate intervals by ECS, conditioning shock with subsequent sham-ECS (corneal electrodes applied, but no current passed), and no conditioning shock and sham-ECS. The outcome of the control group treatments is expected to be very similar to that obtained in pilot studies reported in the preliminary supporting data appended to this application. The data resulting from the proposed experiments will allow for conclusions regarding the dose-response relationship for nicotine sulfate and the rate of memory consolidation, determined from the incidence of reduced retrograde amnesia induced by ECS. Also, the temporal gradients for ECS-induced retrograde amnesia will be determined for nicotine sulfate. Since this

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compound, within the proposed dose range, does not alter the susceptibility of mice to electroshock-induced convulsion, one may therefore assume that any change in the amnesic effect of electroshock is due to a central event rather than a threshold change. The usual temporal gradient for electroshock-induced amnesia has, in our experience, fallen within 1-hr. post-training with a reduced incidence of amnesia occurring as a function of the training electroshock time interval. From preliminary data it appears as though the gradient is appreciably shortened when animals have been pretreated with nicotine sulfate. The results of the proposed studies should conclusively establish the temporal boundaries of the amnesic effect of ECS.

The second series of proposed experiments will concern the effects of nicotine sulfate, to be utilized in the dose range specified above, upon the acquisition and retention of conditioned avoidance behavior as a function of (a) conditioning stimulus intensity (1.0, 2.0, or 3.0 mA) and (b) post-training ECS intensity (5.0, 10.0, or 20.0 mA). The incidence of conditioned response acquisition and the duration of stability of this response has been shown to increase as the conditioning shock intensity is increased, and the incidence of electroshock induced amnesia is increased as the ECS intensity is increased.

In the first experiment of this series animals will be conditioned, utilizing the technique described above and will be given either a foot shock of 1.0, 2.0, or 3.0 mA, applied through the grid floor upon successful completion of the response. Control animals in this situation will be given no conditioning shock. For each of these conditions a post-training electroconvulsive shock will be given at 5.0, 10.0, or 20.0 mA, either immediately following the training trial or at 10, 20, 30, or 60 minutes following the training trial. All animals will be tested for acquisition of the conditioned response as a function of the pre-training dosage of nicotine sulfate (0.25 mg/kg to 2.00 mg/kg), 24 hrs. following the training trial where the conditioning shock was varied. These data, for the effects of the drug upon retention as a function of the conditioning shock intensity, will be available from the non-convulsed control animals for which a saline-injected group will serve as a control for the drug dosage. Data relevant to the issue of post-training ECS intensity and the incidence of retrograde amnesia as a function thereof will be determined from nicotine sulfate and saline-treated groups given these injections one hour prior to the training trial. The testing trial will again be given 24 hours following training, and on the basis of the incidence of retrograde amnesia obtained statements with respect to the temporal gradient for retrograde amnesia as a function of an intensity of the amnesic event, electroconvulsive shock, may be made. On the basis of pilot studies it is anticipated that nicotine sulfate will reduce the temporal gradient for the amnesic effect of electroconvulsive shock, and that such a reduction will be a function of decreased ECS intensity as well.

Another series of experiments will be concerned with the biochemical effects of nicotine sulfate in the dose range which proves to be effective in facilitating memory consolidation, as determined from the experiments outlined above. Those specific biochemical changes with which the experiments will be concerned will deal primarily with the biogenic amines serotonin (5-hydroxytryptamine) and norepinephrine, as well as the substrates to which these amines are related. Since preliminary data has suggested that in the strain of mouse used there are no appreciable changes over time in norepinephrine, this observation will again be verified experimentally and the majority of the chemical determinations to be carried out will concern themselves with serotonin metabolism; in this connection, 5-hydroxytryptophane, the serotonin precursor and 5-hydroxyindoleacetic acid, the serotonin metabolite, will be assayed,

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utilizing spectrofluorometric procedures which have been well standardized in this laboratory. Through these means serotonin concentration can be determined and reliable estimates of serotonin turnover may be obtained. These chemical determinations will be carried out under conditions of both experimental as well as control drug treatment and under conditions wherein electroconvulsive shock is administered or controlled for by sham treatment. It is anticipated that as a result of electroconvulsive shock whole brain serotonin concentration in control animals will be elevated. This hypothesis is based upon previous observations in this (Essman, W.B. Changes in memory consolidation with alterations in neural RNA. Proc. Coll. Int. Neuropsychopharm., 1967, 108-113) and other laboratories (Kato, L., et al. Histamine, serotonin, epinephrine and norepinephrine in the rat brain, following convulsions. Int. J. Neuropsych., 1967, 3, 46-51). It is further hypothesized that the degree to which electroshock elevates brain serotonin will be limited under conditions where nicotine sulfate results in decreased brain serotonin levels as a function of dosage and post-injection time, and that under these conditions either serotonin concentration or serotonin turnover may be statistically correlated with the behavioral data indicating the incidence under these conditions of conditioned response retention; i.e., facilitated memory consolidation, inferred from nicotine sulfate-induced attenuation of electroshock-induced retrograde amnesia, will be positively correlated at those dosages and post-injection times where maximal changes in brain serotonin concentration and/or turnover are observed.

The proposed experiments may be summarized as an attempt to explore the relationship between the effects of nicotine sulfate on amine metabolism in the central nervous system and the process of memory consolidation based upon the hypothesis that nicotine-induced alterations in brain amine levels may account for a nicotine-induced facilitation of memory consolidation.

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Preliminary Supporting Data

Several experiments completed in this laboratory have indicated the feasibility of the experiments proposed. The first of these studies was a series concerned with the acquisition by mice of a simple maze response requiring a choice discrimination in order to escape from a water maze. Groups of animals were given either 0.9% saline, 0.25 mg/kg, 0.50 mg/kg, or 1.00 mg/kg of nicotine sulfate, intraperitoneally. 15 min. following injection all animals were given 4 training trials spaced at 15-min. intervals in a water maze. Their escape times and incidence of errorless performance in the acquisition of the escape task were recorded. When the escape times for all groups of animals were linearly transformed across all trials in order to derive a decrement score indicative of the rate of maze acquisition, the only group reaching statistical significance was that comprised of those mice which were given 1.00 mg/kg of nicotine sulfate 15 min. prior to the first training trial ($t = 1.92$; $p < .05$). A comparison between groups for the incidence of errorless performance throughout the acquisition of the escape response indicated that there was a significant difference between groups in errorless performance and that this significance was largely accounted for by the high incidence of errorless performance among those animals receiving 1.00 mg/kg of nicotine sulfate ($\chi^2 = 4.95$, df = 1, $p < .02$). There was a 55% incidence of errorless performance among animals treated with 1.00 mg/kg of nicotine sulfate, as compared with an average of 28% errorless performance among the other groups in this study. These findings suggested that 1.00 mg/kg of nicotine sulfate exerted a significant effect in facilitating the acquisition of a simple escape response by mice.

Subsequent studies were concerned with the effect of a 1.00 mg/kg dose of nicotine sulfate in relation to the memory consolidation process. In these studies animals were treated with either nicotine sulfate or physiological saline 60 min. prior to being given a single training trial to establish a conditioned avoidance response. The training trial was followed immediately by a 10 mA electroconvulsive shock administered transcorneally for 200 msec.; 24 hrs. following the training trial all animals were tested for the incidence of retention and/or retrograde amnesia. Saline-treated animals showed between 90% and 100% incidence of retrograde amnesia, as defined by the absence of any conditioned avoidance behavior, whereas approximately 66% of the nicotine sulfate-treated animals showed retention of the conditioned avoidance response, with the remaining 34% showing the same retrograde amnesia for the conditioned avoidance behavior, as was shown by almost all of the saline-treated control animals. This finding suggested that the amnesic effect of electroconvulsive shock was either attenuated by nicotine sulfate treatment or that the time interval required for fixation of the memory trace was appreciably shortened through the effects of nicotine sulfate and was thereby less vulnerable to the amnesic effects characteristically exerted by post-learning electroconvulsive shock. The first alternative suggested by these data does not seem to appear dependent upon any alteration in the threshold to convulsion produced by electroshock since, for the dosage used, independent experiments established that for electroshock, varied from 5 through 20 mA, there was no alteration in the incidence of a full clonic-tonic convulsion following such electroshock administration at intervals from 15 min. to 60 min. after injection of 1.00 mg/kg of nicotine sulfate.

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In another study where the interval between administration of 1.00 mg/kg of nicotine sulfate and the training trial, immediately followed by electroshock, was varied from 15 min. through 60 min., the data indicated that only when the compound was administered 60 min. prior to conditioning trial did a significant ($p < .01$) incidence of conditioned response retention occur. These data were consistent with those obtained in the previous study in that comparable results emerged, further

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supporting the observation that nicotine sulfate exerts a peak effect upon memory consolidation when its central action occurs one hr. following administration of 1.00 mg/kg to mice. This observation is supported by some biochemical data in which, for saline-injected animals or mice treated with 1.00 mg/kg of nicotine sulfate at various intervals preceding the removal of brain tissue, whole brain serotonin and norepinephrine were extracted and assayed spectrofluorometrically. Contrary to a recent report (Westfall, T.C., et al. Effect of nicotine and related substances upon amine levels in the brain. Ann. N.Y. Acad. Sci., 1967, 142, 83-100) no changes in norepinephrine were observed as a function of post-injection time up to 60 min. post-injection, whereas whole brain serotonin did generally show a trend toward a decrement as a function of time. These data and the behavioral data previously referred to are summarized in Table 1.

Table 1

Mean Whole Brain Serotonin Concentration and Per Cent Incidence of Conditioned Response Retention after Electroshock for Mice given 0.9% Saline or 1.00 mg/kg of Nicotine Sulfate at Varying Intervals Prior to Conditioning.

Condition	Mean Whole Brain Serotonin	σ	Per Cent Incidence of CR Retention after ECS
0.9% Saline	0.677	0.089	10
Nicotine Sulfate (1.00 mg/kg)			
15 min.	0.676	0.058	10
30 min.	0.688	0.107	20
45 min.	0.655	0.083	25
60 min.	0.640	0.057	66

These data are consistent with a previous suggestion (Essman, W.B. Changes in memory consolidation with alterations in neural RNA. Proc. Coll. Int. Neuropsychopharm., 1967, 108-113) that a reduction in brain serotonin level or pharmacologically-imposed limitations on the rate of serotonin turnover in the brain tend to limit the degree to which amnesic agents or events become effective in imposing a retrograde amnesia, and that these observations may be interpreted as a facilitation of the memory consolidation process whereby the time interval following learning, wherein the memory trace is normally vulnerable to the disruptive effects of known amnesic agents or events, is appreciably shortened by those conditions which tend to lead to a reduction in or limitation on the activity of brain serotonin. It is felt that nicotine sulfate is one such agent which, at the dosage utilized, provides for a wide margin of safety insofar as neurotoxic effects are concerned.

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11. Biographical Sketch:

WALTER B. ESSMAN

Education:

Ph.D. - University of North Dakota, 1957
M. A. - University of North Dakota, 1955
B. A. - New York University, 1954

Experience:

Instructor, Dept. of Neuropsychiatry, U.S. Army Medical Service School, 1958-59.
Director, Psychophysiological Research, U.S. Army Surgical Research Unit, 1958-59.
Senior Post-Doctoral Fellow, Neurophysiology, Albert Einstein College of Medicine, 1959-61.
Research Associate, Dept. of Physiology, Albert Einstein College of Medicine, 1961-62.
Assistant Professor, Queens College, 1962-64.
Associate Professor, Queens College, 1965-66.
Professor, Queens College, 1967 - Present.
Research Fellow, Laboratory of Neurochemistry, Mt. Sinai Hospital, New York, 1964-66.
Research Associate, Laboratory of Neurochemistry, Mt. Sinai Hospital, New York, 1966 - Present.
Lecturer (Neurochemistry), Columbia University, 1964 - Present.

Areas of Major Research Interest:

1. Biochemistry of the central nervous system.
2. Neurochemical correlates of behavior.
3. Memory processes.

Memberships:

American Psychological Association
American Physiological Society
American Society of Zoologists
New York Academy of Sciences
American Association for the Advancement of Science

Honor Societies:

Psi Chi
Phi Delta Kappa
Alpha Pi Zeta
Sigma Xi

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12. Most Recent and Pertinent Publications:

Essman, W. B. and Alpern, H. Single trial learning: methodology and results with mice. Psychol. Rep., 1964, 14, 731-740.

Essman, W. B. Effect of tricyanoaminopropene on the amnesic effect of electroconvulsive shock. Psychopharmacologia, 1966, 9, 426-433.

Essman, W. B. Changes in memory consolidation with alterations in neural RNA. Proc. Coll. Int. Neuropsychopharm., 1967, 108-113.

Essman, W. B. Changes in locomotor activity and brain chemistry following LSD-25 administration in mice. Psychol. Rep., 1967, 20, 124-126.

Essman, W. B. Facilitation of memory consolidation by chemically induced acceleration of RNA synthesis. Proc. XXIII Internat. Congr. Physiol. Sciences, 1965, 470.

Essman, W. B. Modification of neural RNA with amnesic agents and events. Proc. XVIII Int. Congr. Psychol., Moscow, 1966, 20, 65-67.

Essman, W. B. Alterations in memory consolidation as a function of the synthesis and turnover of neural 5-hydroxytryptamine. Seventh Int. Congr. Biochem., 1967, Tokyo.

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